SOME EFFECTS OF RADIATIONS FROM A MERCURY VAPOR ARC IN QUARTZ UPON ENZYMES[1]

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I. Review of Previous Experimentation

Numerous experiments have been performed with the object of determining the effects of radiations from mercury and carbon arcs and from the sun upon enzymes *in vitro*. The results have usually shown agreement, in one feature at least—that light sources which emit ultra-violet rays induce partial or complete inactivation of the irradiated enzyme solutions, depending upon the intensity or irradiation and the duration of exposure. In most of these experiments standardization of certain physical conditions of the procedures has been neglected, with the consequence that certain results have been at variance. The two features which have been most neglected are these: (1) Quantitative measurements of the energy emission of the light sources; (2) The determination of comparative effects of different portions of the spectrum upon the enzymes in question. In most of the published works, energy measurements have been omitted completely, so that it is impossible to review various experiments upon a comparative basis. Moreover, the destructive effects of the radiations have been assigned almost exclusively to the ultra-violet portion of the spectrum, with complete disregard for possible effects of other wave-lengths. It has been fairly well established that the visible rays exert little or no effect, but the influence of infra-red radiations which form a large percentage of the energy emission of arcs has been overlooked. Some of the more recent experimenters have irradiated enzyme solutions in quartz vessels immersed in water baths, but they have made no studies upon the relative effects of the infra-red and ultra-violet wave-lengths.

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In this review of literature, only those experiments which involve ultra-violet radiations will be summarized. It is both irrelevant and unnecessary to dwell upon the effects upon enzymes of other types of radiation: X-rays, radium emanations, etc.

Probably the first biologists to study the effects of light upon enzymes were Downes and Blunt ('79), who found that zymase was inactivated in quartz tubes by the full solar spectrum in the presence of oxygen; they concluded that the injurious effect was due chiefly to the blue-violet portion of the spectrum and that the inactivation process was an oxidation reaction. Fermi and Pernossi ('94), in a study of a number of environmental factors upon enzymes, found that pepsin and trypsin in aqueous solutions are attenuated by long exposure to sunlight, that diastase and ptyalin are but slightly injured by such exposure, and that bacteria exposed to sunlight lost considerable of their proteolytic activity. Green ('97) studied in great detail the action of sunlight and light from an electric arc upon diastase in leaves and in aqueous extracts; he found that both in the tissues and extracts the diastase suffered a considerable loss in activity, but that such injury did not occur when ultra-violet rays were removed by glass filters. In fact, when such filters were used, Green found that an increase in diastatic activity often occurred. He likewise showed that the proteins and perhaps the chlorophyll in leaf cells exert a protective influence for the enzymes against the abiotic ultra-violet rays. Emmerling ('01) contributed further to the study of the effects of sunlight upon enzymes; he found that invertase, maltase, lactase, amylase, and rennet suffered various degrees of attenuation, but that pepsin, trypsin, and emulsin were apparently unaffected. Von Tappeiner ('03) studied the effects of sunlight upon enzymes with and without the addition of fluorescing substances such as Magdala red, chinolin red, and eosin; he found in the case of diastase, invertase, and papayotin that little or no injury occurred when exposure was made without a fluorescing substance, but that when such a material was added, even in so dilute a concentration as 1: 1,000,000, definite attenuation of enzyme activity resulted.

The Schmidt-Nielsens ('04, '08) exposed solutions of chymosin, chymosinogen, and antichymosin of blood serum for short periods to a carbon arc and found that in all cases an exposure of fifteen minutes was sufficient to produce as much as 95 per cent inactivation; when the ultra-violet spectrum was removed by filters no inactivation occurred, and they concluded that the ultra-violet rays were responsible for the damage. Jodlbauer and von Tappeiner ('06) exposed solutions of invertase to sunlight with and without the ultra-violet rays, and found that sunlight without ultra-violet was capable of producing a small amount of injury, which was increased to 80 per cent in ten minutes if a small amount of a fluorescing substance was added. The same authors later ('06a) rayed solutions of invertase with a carbon arc in a water bath to exclude infra-red radiations and found a definite injury when oxygen was present; from this, they concluded that the process was an oxidation reaction. Dreyer and Hanssen ('07) found that the radiations from a mercury vapor arc weakened the enzymes rennet, trypsin, and papayotin; they believed that the ultra-violet radiation was chiefly responsible for this injury, since a glass plate inserted between the arc and the enzymes prevented attenuation. The Schmidt-Nielsens ('08) rayed commercial solutions of rennet in a water bath beneath a mercury vapor arc and found a destruction of activity proportional to the length of irradiation, to the energy given off, and to the dilution, the greater dilutions of the enzyme suffering a greater degree of injury. With the use of special filters they determined that 93 per cent of the injury was attributable to the wave-lengths 200–250 mμ, 4 per cent to 250–313 mμ, and 3 per cent to the visible spectrum. They supposed the destruction process to be monomolecular and to be more or less independent of temperature.

Pougnet ('10) found that in the cumarin plant the characteristic odor is formed after irradiation. This process is dependent upon the activity of enzymes which were thus shown to be uninjured by the radiations. Pougnet ('11) found a similar condition in the production of odor in vanilla pods. Stassano and Lematte ('11) exposed suspensions of bacteria to a mercury vapor arc beneath a thin film of water to remove heat rays and found that bacteria killed by such exposure produced agglutination which was only slightly less pronounced than that caused by living bacteria; hence they concluded that the inactivation of enzymes contributed little if any to the killing of bacteria by ultra-violet rays. Chauchard and Mazoué ('11) rayed solutions of malt amylase and yeast invertase with a quartz mercury vapor arc in separate and in mixed solutions and found that the amylase at the end of one hour's exposure was only 10 per cent as active as unrayed solutions, but that invertase had lost only 45 per cent of its activity. They concluded that it was possible to destroy amylase completely and have some active invertase left if a mixture of the two were rayed for a sufficient period of time. Agulhon ('12) studied the effects of radiations from a mercury arc upon sucrase, malt amylase, pancreatic amylase, pepsin, emulsin, and rennet, and reported that all of these enzymes suffered some degree of attenuation from wave-lengths shut out by glass but transmitted by quartz—in other words, the injury was caused by ultra-violet rays, with the visible rays contributing practically nothing to this effect. Burge ('17), in attempting to discover the mechanism by which ultra-violet rays kill protoplasm, found that such organisms as *Bacillus liquefaciens*, *B. prodigiosus*, etc., could be killed by ultra-violet rays without any apparent decrease in the power of their extracted enzymes to liquefy gelatin.

In 1923, Ludwig Pincussen, at the City Hospital of Urban, Berlin, began the publication of a series of papers entitled "Fermente und Licht," which reported a number of experiments conducted by him and his associates upon that subject. The first paper ('23) described the effects of sunlight and of a quartz mercury arc upon solutions of malt diastase as follows: 1. Sunlight had little or no effect upon the enzymes. 2. The mercury arc produced strong inactivation. In the summary of his paper, Pincussen mentions certain laws which seem characteristic of the action of abiotic radiations upon enzymes: "Die Wirkung des Lichtes auf die Malzdiastase ist äbhangig von der Verdünnung, von verschiedenen Begleitkörpern und vor allem von der Reaktion. Die grösste Lichtschädigung erfolgt bei einer Reaktion die als die optimale für die Fermentwirkung anzusehen ist. Es scheint demnach, dass das Fermentmolekül bei seiner optimalen Wirkungsreaktion besonders labil gegenüber der Lichtenergie ist." Furthermore, Pincussen writes "Es scheint dass in gewissen Grenzen die Temperatur ohne Einfluss auf eine mögliche Lichtschädigung ist," thus omitting possible effects of the infra-red radiation from the arc upon the enzyme solutions.

Pincussen and Kato ('23) exposed solutions of soy-bean urease to sunlight and to the radiations from a quartz mercury arc and found that both strong sunlight and the arc radiations produced injury which was greatest at the optimum pH of the enzyme. Essentially the same results were found in a somewhat more extensive paper by Pincussen and Kato published later ('23a). Pincussen and di Renzo ('24) continued the work on diastase in various concentrations; they found that the injury produced by a mercury vapor arc decreased with increasing concentration of the enzyme in solution and that the inactivation reaction is monomolecular. Pincussen ('24) rayed malt and Taka-diastase and found the Taka-diastase somewhat more easily inactivated by ultra-violet than the malt-diastase. He discovered that when certain salts were added to the enzyme solutions, the degree of injury caused by the ultra-violet rays was noticeably decreased. He ('24a) continued his studies upon the protective action of salts and found that iodine salts particularly were instrumental in causing protection against ultra-violet rays. With Klissiunis ('24) he studied further the protective action of iodine salts, potassium and sodium iodides, and in addition the iodides of alkali earths, lithium iodide, rubidium iodide, etc., upon solutions of trypsin. They found that the addition of any of these salts decreased considerably the injury produced by light in the enzyme solutions. Pincussen and Seligsohn ('26) rayed solutions of blood catalase at 10 cm. from a mercury arc (with no protective measures against infra-red) and found that inactivation followed the laws for other enzymes: the proportionality of inactivation to dilution and optimum pH, and the protective action of salts, reported previously for diastase and trypsin. Pincussen ('26) reported additional experiments upon diastase, in which he described a "reactivation" effect in the case of purified malt diastase. When diastase solutions which had been injured in varying degrees by exposure to a mercury arc were mixed with unrayed solutions, the mixtures showed greater diastatic activity than the sum of the activities of the separate solutions. This reactivation is the more intense the greater is the injury to the rayed solutions; when the injury is slight there is little or no reactivation; further, the reactivation is more striking the greater are the quantities of rayed diastase in the mixture.

Pincussen and Kumanomidoh ('28), in further work upon the addition of salts in relation to light inactivation, discovered a protective action of the chlorides of K, Ca, Mg, Li, and Pb. The order of protectivity varied with the different diastases used —salivary, malt, and Taka—and also with the concentrations of the salts and of the enzyme solutions. In general, the potassium salts seemed to offer the least protection, the calcium salts the greatest. Pincussen and Uehara ('28) rayed solutions of pepsin in a water bath with a mercury vapor arc and found definite inactivation which was greatest at pH 1.15, which is near the optimum for peptic activity. At pH 1.93 and pH 2.28 there was no significant difference between the activities of rayed and unrayed pepsin solutions. Pincussen and Hayashi ('28) used serum lipase from rabbits and guinea pigs, which was exposed at twenty centimeters from a mercury arc (again with no consideration of infra-red) and which suffered definite injury chiefly at acid pH values. When accompanying proteins were removed by precipitation with ammonium sulphate the injury was greater. The injury was practically nil at alkaline pH values. Pincussen and Kambayashi ('28) studied the effects of sensitizing agents, particularly eosin, upon the inactivation of Taka-diastase by ultraviolet and found that the addition of the agent in no case increased the degree of injury produced by a mercury arc, a result contradictory to earlier findings, and that with increasing concentration of the sensitizing agent there was a decrease in the amount of injury produced by light. In all cases the greatest inactivation occurred at the optimum pH, with or without the addition of the fluorescing agent. Pincussen and Oya ('29) studied the effects of light from a mercury arc upon Taka-diastase solutions in water baths at different temperatures. They found greater inactivation at higher temperatures (50° C.) than at lower ones (16°). They found no reactivation phenomena. Pincussen and Oya ('29a) rayed solutions of Taka-diastase with a mercury arc and studied the lecithin-hydrolyzing power of the enzymes lecithase and phosphatase, which are invariably present in the diastase preparation under such treatment. They found a decrease in the activity of these two enzymes; the above-mentioned laws held true here again. They ('29b) found a great degree of inactivation of milk aldehydrase after an irradiation of one hour with a mercury vapor arc in the presence of oxygen. Pincussen and Roman ('30) found that the succinodehydrogenase of horse muscle was strongly injured in the pH region 6.0–8.0 by a fifteen-minute exposure to a quartz mercury arc; a lesser injury was produced by one hour's exposure to visible light.

Thompson and Hussey ('25–'26) rayed aqueous solutions of pepsin in a water bath above a mercury vapor arc and found definite inactivation of the enzyme. They concluded that the process was that of a monomolecular chemical change. They also decided, from that fact that .5 cm. of water separated the enzyme tubes from the arc, that "it would seem that the effective radiations are those in the ultra-violet region of the spectrum." In view of the fact that .5 cm. of water removes only a portion of the infra-red radiation, this assumption might be questioned.

Tauber ('30) found that exposure to direct sunlight does not injure urease; if, however, eosin is added, definite injury occurs. The radiations from a quartz mercury arc he found likewise to be inhibitory to the activity of urease; eosin increased the injurious effect. Thompson and Hussey ('31) rayed amylase solutions in a water bath with a mercury vapor arc and found inactivation which followed the course of a monomolecular radiochemical change and which they attributed solely to the ultra-violet wave-lengths in the arc spectrum. Pace ('31) studied the effects of radiations from a mercury arc upon solutions of trypsin and enterokinase which had been previously partially inactivated by heat; with irradiation, only further inactivation occurred; with visible light only, there was no further effect upon the enzymes. Pace made no provisions in his work for removing infra-red radiation.

Thus the uncontrolled features of these experiments, emphasized in an opening paragraph, are made obvious: (1) The failure to consider the energy output of the light source; (2) The neglect of the possibility of enzyme injury by infra-red radiation. As mentioned in the review of literature, a few experimenters have taken precautions for the removal of infra-red but in no work so far has a comparative study of the effects of ultra-violet and infra-red been completed; (3) The almost complete lack of study of the effects of ultra-violet radiation upon enzymes *in vivo;* too often the results of test-tube experiments have been utilized to explain the effects of radiations upon organisms without attempting to study the effects upon enzymes in tissues.

II. Statement of the Problem

It was the object of the experiments reported in this paper, first, to study the effects of radiations from a quartz mercury vapor arc upon two commonly used enzyme preparations, Taka-diastase and Difco invertase, with respect to the comparative effects of infra-red and ultra-violet portions of the spectrum upon them, with careful measurements of the energy output of the irradiation sources; second, to study the effects of the full ultra-violet spectrum, plus the visible spectrum, upon enzymes in plant tissues, with a view to determining whether or not the injury to these tissues is caused by injury to enzymes.

These two groups of experiments will be designated respectively as Series I and Series II.

III. Experimental Methods and Results

SERIES I

In this group of experiments, Taka-diastase and Difco invertase were used. In both cases, aqueous solutions of the enzymes were rayed in 2-mm. layers in open Petri dishes at 10 inches from a quartz mercury vapor arc, a Burdick lamp operated at 75 volts and 6 amperes. Experiments with infra-red rays present and with infra-red rays excluded were conducted in parallel. For the removal of the infra-red rays from the spectrum, a quartz water cell, consisting of a chamber with a 6-inch square of quartz 2 mm. thick for the bottom and a 1.5-cm. layer of water, was inserted midway between the arc and the enzyme solutions. Such a filter removed the greater part of the infra-red radiation and transmitted without appreciable diminution of energy the ultra-violet wave-lengths. Hence, with the water cell in place it was possible to study the effects of the visible and ultra-violet spectra together, without the infra-red; since it has been conclusively demonstrated that the visible spectrum has a negligible effect upon enzymes, the results obtained with the water cell may be attributed exclusively to the ultra-violet rays. When the water cell is not inserted the combined effect of ultra-violet and of infra-red is obtained, from which by simple subtraction one can determine the extent to which infra-red produces injury and thus draw a comparison with the effects of ultra-violet alone, unless the combination of infra-red and ultra-violet produces a special effect.

Intensity measurements were made by means of a Leeds & Northrup type P reflecting galvanometer No. 2239 with a sensitivity of .7 microamperes, and two Cenco linear thermopiles arranged in parallel. A carbon filament incandescent lamp from the United States Bureau of Standards, standardized to give a radiation of 86.2×10^{-8} watts per sq. mm. of receiving surface at 2 meters when lighted at .4 amperes and 99.5 volts, was used as a basis for computing the radiant energy given off by the arc. The intensity measurements for Series I are as follows:

At a distance of 10 inches from the arc, with the quartz water cell in place: 5025.0×10^{-8} watts per sq. mm., or 1202.15×10^{-8} gm. calories per second per sq. mm. Without the water cell, at the same distance: $45{,}225.0 \times 10^{-8}$ watts per sq. mm. or $10{,}826.17 \times 10^{-8}$ gm. calories per second per sq. mm. It is evident that at this distance from the arc the energy falling upon a unit of area is composed of about 12 per cent ultra-violet and visible and about 88 per cent infra-red.

1. Taka-diastase.—Taka-diastase was rayed in .5 per cent aqueous solution in 10-cc. portions with and without the water cell before the arc, as described above. In all cases the period of exposure was 30 minutes. At the end of the irradiation period, distilled water was added to make the solutions up to 10 cc. to make up for evaporation during the irradiation. Then the activity of the rayed sets and of the unrayed (controls) was determined by Appleman's ('11) modification of the Wohlgemuth ('08) iodine method for diastase, which is described below.

Three sets of 10 test-tubes each were arranged in test-tube racks and were designated *a*, *b*, and *c*, which referred respectively to the solutions unrayed, rayed with the water cell, and rayed without the water cell. In each set the tubes were numbered in units from 1 through 10. Ten cc. of a 2 per cent Lintner soluble starch solution were placed in each tube, and the 3 sets were immersed in an ice bath until the temperature of the starch solution in the tubes was the same as that of the bath; this was done to prevent enzyme action until all tubes were inoculated and could be set in the incubator. Then, in each set 1 cc. of the enzyme solution was placed in the tube numbered 10, .9 cc. in tube 9, .8 cc. in tube 8, and so on, down through tube 1, which received .1 cc. of the enzyme solution.

After the enzyme solutions had been added to the proper tubes the tubes were all set away in an incubator adjusted to 39° C., where they remained for 45 minutes. Following this incubation period, the tubes were again immersed in an ice bath until the temperature of the enzyme-substrate mixtures was the same as that of the bath, thus preventing further enzyme action. Then 2 cc. of the mixture were pipetted out from each tube into a glass vial bearing a number corresponding to that of the tube, 5 cc. of distilled water and 2 drops of iodine-potassium iodide solution (Meyer's) were added, and then color changes in the tubes in the 3 sets were noted. A yellow color indicated complete hydrolysis of starch; a reddish-yellow, a complete disappearance of starch but the presence of some dextrine; a red-violet, not quite complete disappearance of starch; and a definite violet color, the presence of considerable quantities of starch. The first tube in the descending series which showed a violet tinge was the index for comparing diastatic activity, since it represented the lowest limit of enzyme activity. The activity of the enzyme is expressed as the number of cc. of the starch solution which 1 cc. of the enzyme solution could hydrolyze during the incubation. For example, if tube 4, containing .4 cc. of enzyme solution, had the first tinge of violet in the descending series (that is, has practically entirely hydrolyzed the starch in that tube), then 1 cc. of the enzyme solution under the same conditions would hydrolyze $\frac{1.0}{.4}$ or 2.5 times the same quantity of starch solution. If 10 cc. of starch solution is used as the substratum, then the quantity of solution which would be hydrolyzed by 1 cc. of the enzyme solution would be 10 × 2.5 or 25 cc. This figure is taken as the diastatic power of that enzyme under the specified conditions. The conditions and the diastatic power are usually expressed thus:

$$D\frac{T}{t} = N$$

wherein D is the diastatic power, T the temperature of incubation, t the duration of incubation and N the number of cc. of starch solution hydrolyzed by one cc. of the enzyme solution.

The Wohlgemuth method is perhaps not as fine as the copper method of measuring amyloclastic activity, but as Sherman, Kendall, and Clark ('10) write: "Although it is not quite as accurate as the gravimetric copper method, it is easier to perform and has the theoretical advantage of marking the completion of a fairly definite step in the digestive process, whereas the copper reduction method measures the amount of a substance or substances produced by successive steps through intermediate products which are but imperfectly known."

TABLE I

TAKA-DIASTASE

(T, 39°, t, 45 min.)

Experiment number	Set A Unrayed D	Set B Rayed with water cell D	Set C Rayed without water cell D
1	100.0	14.3	10.0
2	100.0	16.7	10.0
3	100.0	16.7	10.0
4	100.0	16.7	10.0
Average	100.0	16.1	10.0

The results of the Taka-diastase experiments are presented in table I.

From the table it is evident: (1). That considerable injury occurs in Taka-diastase with the water cell in place, and that such injury is caused by ultra-violet radiation; (2). That greater injury occurs when the water cell is removed; that is, the infra-red exerts an appreciable injurious effect in addition to that of the ultra-violet rays.

2. Difco invertase.—The Difco standardized invertase solution was diluted in equal parts with distilled water and then rayed in open Petri dishes under exactly the same conditions as those employed in the experiments upon Taka-diastase. The experimental sets were designated in the same way: A, the unrayed set; B, the set rayed with the water cell; C, the set rayed without the water cell.

After the irradiation, 20 cc. of the enzyme solution were added to 150 cc. of 20 per cent sucrose solution, previously adjusted to pH 4.6 with .1 N sodium acetate. The activity of the invertase solutions was determined by the polarimetric method as described by Waksman and Davison ('26). The rotation of each invertase-sucrose solution was determined immediately after the addition of the invertase and then from time to time thereafter to determine the time required to reach the end point. During this interval the solutions were kept in an incubator at 36° C. The periods of time required to bring the solutions to the same end point were taken as the indices of enzymatic activity (Waksman and Davison, '26, p. 177). The results of the invertase experiments are presented in table II. The table shows that the same

TABLE II

DIFCO INVERTASE

(t, time in minutes required to reach end point)

Experiment number	Set A Unrayed t	Set B Rayed with water cell t	Set C Rayed without water cell t
1	30.0	35.7	41.1
2	32.0	39.0	46.0
3	30.0	36.0	42.0
Average	30.66	36.90	43.03

results which obtained in the diastase experiments were evident here: there is definite injury caused by the ultra-violet radiation, which is significantly increased when the infra-red rays also are present to act upon the enzyme.

Expressing the value of the unrayed set average as 100, we find the values of sets B and C to be respectively 89.00 and 70.75.

SERIES II

The experiments described in this section were designed to study the effects of the ultra-violet radiations from a mercury vapor arc upon enzymes in plant tissues, with a view of obtaining information concerning the physiologic mechanism by which such radiations damage and kill vegetable tissues. The enzymes studied were amylase, invertase, peptase, and catalase. The plants used were "Bonny Best" tomatoes, red kidney field beans, and a strain of *Fusarium Lini* Bolley, kindly provided by Mr. W. E. Brentzel of the North Dakota Agricultural Experiment Station. As pointed out in the review of literature, the effects of ultra-violet rays upon enzymes *in vivo* have been studied in only two or three cases, and in those (with the exception of Green's work) the studies have been limited to the enzymes of bacteria. So far as the author is aware, this is the first paper in which studies of the effects of ultra-violet rays upon several enzymes in the tissues of multicellular plant forms are reported in detail.

A. BEANS AND TOMATOES

The bean and tomato plants were grown in individual 4-inch pots to a height of about 20 cm. when irradiation was begun. The plants were rayed 3 minutes daily for 7 days at a distance of 50 cm. from the arc. A quartz water cell containing 1.5 cm. of distilled water was placed a short distance below the arc to remove the infra-red rays from the radiations which reached the plants. Intensity measurements, made as described in Series I, showed 1256.25×10^{-8} watts per sq. mm. or 302×10^{-8} gm. calories per sq. mm. per second.

In both the beans and tomatoes the irradiation produced serious damage in the plants. The upper epidermises of the leaves were badly burned and the leaves showed the bronzing and curling typical of ultra-violet injury. At the end of the 7-day period, the enzyme determinations were made. In all cases the measurements of activity were made on fresh tissue.

1. Amylase.—Ten gms. of fresh leaf tissue, after the removal of petioles, were ground with 10 gms. of quartz sand in a mortar for 3 minutes. Five cc. of distilled water were added to the mixture and the juice was pressed out through two layers of cheesecloth, after which it was centrifuged at 2000 r.p.m. for 4 minutes to remove solid materials from the supernatant liquid. The activity of the amylase in the juice was then determined by the Wohlgemuth method as described in Series I. One cc., .9 cc., .8 cc., and similarly descending quantities of the juice were placed in the properly marked test-tubes, each of which contained 10 cc. of chilled 1 per cent Lintner soluble starch solution. As a preservative, .5 cc. of toluol was added to each tube, after which the tubes were stoppered and set away in an incubator maintained at 39° C. It was found by preliminary trials that incubation periods of 6 hours for beans and 24 hours for the tomatoes were the optima. After the incubation the tubes were again chilled with ice and 2 cc. of liquid from each tube were pipetted off into vials marked to correspond with the test-tubes. Five cc. of distilled water and 2 drops of iodine solution were added to each vial, and color comparisons made. The results of the amylase experiments are presented in table III.

TABLE III

AMYLASE

(T, 39°; t, 6 (beans)—t, 24 (tomatoes))

Experiment number	Beans		Tomatoes	
	Unrayed D	Rayed D	Unrayed D	Rayed D
1	16.6	50.0	16.6	25.0
2	16.6	33.3	16.6	25.0
3	16.6	33.3	16.6	20.0
Average	16.6	38.8	16.6	23.3

Choosing 100 arbitrarily as the value of the activity of the amylase in the averages of the unrayed groups, we find the following comparisons:

Beans—Unrayed, 100.00; rayed, 258.40.
Tomatoes—Unrayed, 100.00; rayed, 155.37.

The figures show conclusively that there is a definite and significant increase in the amyloclastic activity of the juice of tomato and bean plants which have been badly injured by ultra-violet radiation.

2. Invertase.—In these experiments the plant juice was extracted and prepared in the same fashion as in the amylase experiments. The activity of the enzyme was determined by adding 5 cc. of the juice to 100 cc. of 15 per cent sucrose solution and incubating for 12 hours at 39° C. At the end of this period, 10 cc. of the mixture were added to 30 cc. of Fehling's solution which was just at the boiling point, and the mixture was allowed to boil for exactly 3 minutes. The tubes were then cooled and the cuprous oxide precipitate was determined quantitatively by titration with potassium permanganate as follows: The solutions were filtered and the residue of cuprous oxide was dissolved in Bertrand's solution (20 per cent sulphuric acid saturated with ferric sulphate), and this solution was then titrated with twentieth-normal potassium permanganate solution. The number of cc. of the permanganate solution required to give the copper-Bertrand solution a faint permanent violet tinge was taken as the index of enzyme activity.

In the invertase experiments, blank solutions were arranged which contained 5 cc. of the plant juice and 100 cc. of distilled water substituted for the sucrose solution. The copper in these solutions was determined just as in the solutions containing sucrose; in this way the quantity of invert sugars present in the plant extract was determined. This value was then subtracted from that obtained from the enzyme-sugar solution to give the actual amount of reducing sugars formed in the latter as a result of invertase activity.

The results of the invertase experiments are presented in table IV.

Using the value 100 as an expression of invertase activity in the averages of the unrayed sets, we find the activity in sets to be:

Beans—unrayed, 100.00; rayed, 124.29.
Tomatoes—unrayed, 100.00; rayed, 125.99.

TABLE IV

INVERTASE

(cc. of potassium permanganate)

Experiment number	Beans		Tomatoes	
	Unrayed	Rayed	Unrayed	Rayed
1	8.6	11.0	11.5	15.0
2	8.4	9.8	11.2	14.5
3	8.2	9.9	14.1	16.9
Average	8.4	10.23	12.26	15.46

Thus a distinct increase is demonstrated in the activity of invertase in the rayed plants.

3. Peptase.—The term "peptase" is used here as proposed by Fisher ('19) to include those enzymes whose activity is peptoclastic; the term is synonymous with the name "ereptase" proposed by Vines at an earlier date. The method of determining the activity of the peptase was that of Sorensen ('08) as reported by Fisher ('19), by means of which the degree of hydrolysis of the substratum is determined from the number of free hydroxyl groups formed.

The measurements of activity were made as follows:

Ten grams of fresh leaf material, without petioles, were ground vigorously with 10 g. of quartz sand in a mortar for 3 minutes, after which the mixture of pulp and sand was added to a solution of 5 gms. of Witte's peptone in 250 cc. of distilled water. The solution was covered with toluol as a preservative and placed in an incubator at 38° C. for 3 days. At the end of that time, the solutions were filtered rapidly through Buchner filters by vacuum, and the residue was washed with distilled water until the original solution and the washings made up a volume of 400 cc. Forty cc. of the filtrate were removed to a small flask and decolorized by shaking with 1 g. of alumina cream. After standing for 4 minutes, the solution was filtered and the residue washed in distilled water to make a volume of 65 cc. of filtrate. To this were added 15 cc. of a solution of thymolphthalein made up as follows: 50 cc. of 40 per cent formaldehyde, 25 cc. of absolute alcohol, and 10 cc. of thymolphthalein solution (.5 g. in 1000 cc. of 93 per cent alcohol).

The formaldehyde neutralized the free amino groups of the amino acids produced, forming methylene compounds; the carboxyl groups were then determined by titrating with fifth-normal barium hydroxide solution, the thymolphthalein acting as an indicator. The barium hydroxide solution was added to the decolorized filtrate until a distinct blue color developed.

It was necessary to apply corrections to compensate for the amino acids already present in the tissues and in the peptone. For this the tissue was ground as described above and added to 250 cc. of distilled water without peptone. After 3 days of incubation the determination was applied as above, the obtained value representing the relative amino acid content of the tissue. To determine the amino acids originally present in the peptone, a flask containing 250 cc. of 2 per cent peptone solution only was incubated for the usual period of time and then its amino acid content determined by the titration method. These two values—the amino acid content of the tissue and that of the peptone—were subtracted from those obtained from the tissue-peptone mixtures; the resultant figures represented the actual amount of amino acids formed as a result of the hydrolysis of the peptone by the peptase. The amount of the hydrolysis can be calculated as milligrams of nitrogen by multiplying the number of cc. of fifth normal barium hydroxide solution required to produce a blue color by 2.8. The results of the peptase experiments are shown in table v.

TABLE V

PEPTASE

(Milligrams of nitrogen liberated)

Experiment number	Beans		Tomatoes	
	Unrayed	Rayed	Unrayed	Rayed
1	165.0	198.6	103.6	140.0
2	176.4	201.6	123.2	148.4
3	182.0	207.2	112.0	134.4
Average	174.46	202.53	112.60	140.93

Expressed on the basis of 100 as the value of the controls, the following figures for the averages are obtained:

Beans—unrayed, 100.00; rayed, 115.44.
Tomatoes—unrayed, 100.00; rayed, 124.01.

Thus, the peptoclastic activity of the juice of tomato and bean plants which were damaged by ultra-violet rays seems consistently higher than that of unrayed plants.

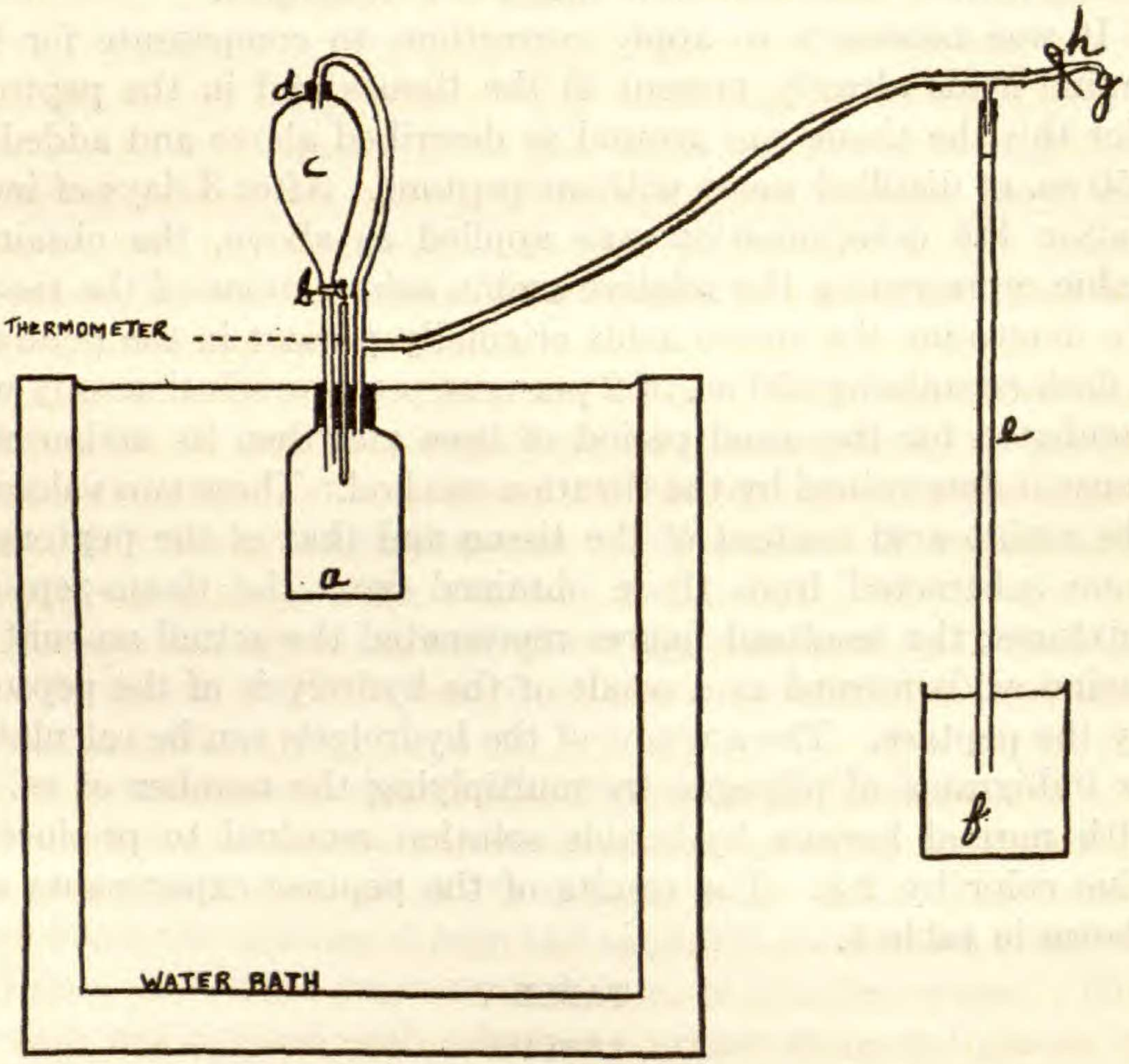

Fig. 1. Apparatus used in determining catalase activity: *a*, reaction bottle; *b*, stopcock; *c*, funnel; *d*, stopper; *e*, burette; *f*, water reservoir; *g*, rubber tube; *h*, stopcock.

4. Catalase.—The catalase activity of the juices of the rayed and unrayed plants was determined by the usual hydrogen peroxide method in a modified Appleman ('10) apparatus.[1] The apparatus used in these experiments differs from that devised by Appleman in that it has but one burette containing a column of water supported by atmospheric pressure on the open barometer principle as the volumeter, rather than the two burettes of liquid employed by Appleman. With this modification it is possible to make more accurate readings, since the oxygen released in the decomposition of the peroxide is released into a partial vacuum; it does not suffer compression as a result of having to displace the weight of a water column in addition to atmospheric pressure as is the case in the Appleman apparatus.

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[1] The modified apparatus was devised by Mr. F. Lyle Wynd, of the Shaw School of Botany, and by the author.

Seven grams of leaf tissue were ground for 1½ minutes in a chilled mortar with an equal weight of quartz sand and a little powdered calcium carbonate to neutralize the organic acids released by the crushing of the cells. The pulp was then pressed through two layers of cheese-cloth, and 1 cc. of the expressed juice was placed in the reaction bottle (*a*) of the apparatus, which had previously been immersed in a water bath kept constant at 19° C. Then 1 cc. of cold distilled water was added and the stopcock (*b*) closed. Six cc. of 3 per cent C. P. hydrogen peroxide were placed in the funnel (*c*), the stopper (*d*) tightly inserted in the top of the funnel and the water sucked up into the burette (*e*) from the water reservoir (*f*) by suction applied at the rubber tube (*g*). The tube (*g*) was then closed with a stopcock (*h*) and the funnel stopcock (*b*) was opened, allowing the peroxide to run into the reaction bottle (*a*). The evolution of oxygen began immediately, and the water column in the burette immediately began to fall. After the admission of the peroxide to the reaction bottle, the bottle was shaken vigorously and constantly by hand. The evolution of oxygen was allowed to proceed for 2½ minutes, at the end of which the total volume of gas evolved was read on the burette. All glassware, pipettes, mortars, etc. used in the work were chilled in an electric refrigerator before use, since it has been shown that catalase begins to decompose at temperatures slightly above 20° C.

The results of the catalase experiments upon beans and tomatoes are given in table VI.

With the values expressed on the basis of 100 for the unrayed averages, the following comparative figures result:

Beans—unrayed, 100.00; rayed, 143.18.

Tomatoes—unrayed, 100.00; rayed, 149.74.

The figures show that the activity of catalase in the rayed plants is definitely and considerably greater than that in the unrayed plants, a situation comparable to that obtaining in the case of other enzymes tested in these experiments.

TABLE VI

CATALASE

(Cc. of oxygen released)

Experiment number	Beans		Tomatoes	
	Unrayed	Rayed	Unrayed	Rayed
1	13.5	20.0	25.5	37.8
2	16.4	24.4	22.5	34.8
3	15.9	21.2	22.9	33.6
Average	15.26	21.86	23.63	35.4

B. FUSARIUM LINI BOLLEY

For the experiments with this fungus the following culture medium was used:

Magnesium sulphate	1 g.
Calcium phosphate (primary)	1 g.
Potassium nitrate	8 g.
Bacto-peptone	2 g.
Glucose	15 g.
Lintner soluble starch	5 g.
Distilled water	1000 cc.

The fungus was grown in 50 cc. of this medium in 150-cc. quartz flasks. After inoculation the flasks were kept in an incubator at 31° C. for 5 days, a sufficient period to produce a light film of mycelium on the surface of the culture fluid. After this the flasks were set at an angle of 45° in a horizontal rack and were rayed at 10 inches from the arc through a quartz water cell as described in Series I, the same intensity values obtaining in both cases. Exposure periods of varying length were employed in order to find the minimal time which would result in the death of the fungus. After these exposures, the cultures were allowed to remain untouched for an hour. Then transfers were made from them to fresh sterile culture solutions. As long as such transfers resulted in growth, it was obvious that the rayed mycelia were alive. When, however, no growth appeared in the transfers, it was assumed that the mycelium had been killed by the irradiation. In these experiments, under the conditions mentioned above, an exposure of 12 minutes, with vigorous shaking of the rack at 3-minute intervals, was sufficient to kill the mycelium. After the irradiation the cultures were allowed to stand for an hour before the tissue was prepared for the enzyme tests.

The mycelium mats, torn apart by needles, were poured on to Buchner funnels, washed with several volumes of distilled water, and the excess water removed by pressing the tissue firmly between pieces of filter-paper. The mycelium was then placed in a quantity of 95 per cent alcohol 6 times its own volume and allowed to remain in it for 12 minutes, after which it was placed into a similar volume of acetone, in which it was kept for 8 minutes. Then it was placed in a second volume of acetone for 2 minutes, and after the removal from this bath, was spread out on filter-paper to dry at room temperature. The dried flakes of mycelium were then ground to a fine powder in a mortar. The powder was then placed in tightly stoppered bottles for storage. This powder was used for the determination of amylase and peptase activity.

1. Amylase.—Nine cc. of distilled water were added to 1 gram of the fungus powder in a test-tube, which was then placed in a refrigerator for 12 hours. The juice was then squeezed through double layers of cheese-cloth from the powder-water mixture and was tested by the Wohlgemuth method as described in Series I. The test-tubes containing the starch-enzyme mixtures were incubated for 24 hours at 39° C., after which the iodine test was applied. The results with the amylase experiments together with those of peptase are given in table VII.

2. Peptase.—The activity of peptase was determined by the Sorensen method as described elsewhere in this paper. One and one-half grams of the fungous powder were added to 250 cc. of the 2 per cent Witte's peptone solution in the rayed and unrayed sets, and the determinations made by the thymolphthalein method after an incubation period of 3 days. Blank solutions of powder only and of peptone only were tested as in the work on tomatoes and beans in order to determine the actual amount of amino acids formed in hydrolysis. The results of the peptase experiments are shown in table VII.

TABLE VII

F. LINI

Experiment number	Amylase D		Peptase Mg. of N formed	
	Unrayed	Rayed	Unrayed	Rayed
1	14.3	16.7	112.7	121.0
2	14.3	16.7	104.1	110.6
3	14.3	14.3	100.2	106.8
Average	14.3	15.9	105.6	112.8

Expressed on the basis of 100 as the value of the averages of the unrayed groups the following numerical comparisons can be made:

Amylase—unrayed, 100.00; rayed, 111.09.
Peptase—unrayed, 100.00; rayed, 106.02.

Hence it seems that there is a slight but consistent increase in the activity of amylase and peptase in the irradiated fungi. The question may be advanced as to whether or not the increases are significant, since they are so small; if these apparent increases are not truly significant, the experiments show at least that the enzymes suffer no decrease in activity when the tissue is killed by ultra-violet rays.

III. DISCUSSION

The inactivation of enzymes *in vitro* by ultra-violet rays has led to the supposition by some physiologists that the injury or death of tissues caused by such rays is attributable in part at least to this inactivation of enzymes. A few reports have been made upon the effects of ultra-violet rays upon enzymes in living plant tissue: those of Pougnet, Stassano and Lematte, Burge, and Green. In so far as the author has been able to determine, these are the only experiments which have investigated the effects of ultra-violet rays upon enzymes in plant tissues.

The results of the experiments reported in this paper which have involved the study of a greater number of enzymes in more complex tissues agree with those of the above-mentioned earlier works in regard to this fact: namely, it is possible to produce advanced injury (beans and tomatoes) or death (*Fusarium Lini*) in plant tissues without injury to the enzymes contained by those tissues. From this work the assumption may be made that the injury or death of living protoplasm as a result of ultra-violet irradiation is to be ascribed to physiological disturbances other than the inactivation of enzymes.

The present work presents another interesting discovery: that in some cases the activity of enzymes in tissues injured or killed by ultra-violet rays is greater than that of enzymes in healthy, non-irradiated tissues. The possibility suggested itself that a difference in dry weight or in pH of the rayed and unrayed tissues might account for the difference in enzyme activities. Hence, a number of dry-weight and pH determinations of leaf tissue and extracts were made; the results are presented in table VIII.

TABLE VIII

DRY WEIGHT AND pH DETERMINATIONS

Sample no.	Beans				Tomatoes			
	Rayed		Unrayed		Rayed		Unrayed	
	% dry of wet wt.	pH	% dry of wet wt.	pH	% dry of wet wt.	pH	% dry of wet wt.	pH
1	12.0	5.80	12.4	5.80	12.3	6.18	10.3	6.18
2	13.0	5.75	10.1	5.80	11.5	6.18	11.5	6.10
3	12.1	5.81	11.4	5.65	13.2	6.20	12.1	6.18
4	12.2	5.80	10.2	5.85	11.9	6.18	10.7	6.18
5	10.7	5.80	11.8	5.80	12.1	6.10	11.6	6.15
6	11.6		10.9		11.4		10.8	
7	11.4		11.2		10.3		10.1	
8	12.1		11.7		13.5		11.2	
9	11.2		10.1		11.9		10.4	
10	11.0		10.8		12.3		12.0	
Av.	11.83	5.79	10.06	5.78	12.04	6.16	11.07	6.15

The table shows a slightly greater dry weight in the rayed sets both in the cases of the tomatoes and beans. The differences, however, are so small that the increased activity of the enzymes in the rayed sets can hardly be explained on this basis. The results of the pH determinations show agreement with those of Eltinge ('28), who found no pH differences among rayed and unrayed plants. On the basis of these measurements, then, it must be concluded that the increased enzyme activity is not a result of differences in dry weight or in the pH of tissue extracts.

It was suggested that there might be other secondary ways in which the radiations could have induced such differences in enzyme activity. It is known that in various types of injury to tissues the activity of enzymes, particularly of respiratory enzymes and catalase, is increased. The accelerated activities obtained in these experiments may be such a reaction—merely a stimulation due to tissue injury and not at all a specific reaction to ultra-violet rays. Such an explanation, however, would hardly account for the enormous stimulation obtained in some cases, for example, bean amylase, in which the activity in the rayed tissue is more than 2½ times that in the controls. In such cases, something akin to Pincussen's ('26) reactivation phenomenon may occur. A portion of the enzyme in the tissue may be in a more sensitive condition than another portion and may suffer partial or complete inactivation as a result of irradiation, whereas the second portion may be completely protected by cell proteins, chlorophyll, etc. against the lethal rays. The injured enzyme substance may then through subsequent contact with the uninjured portion experience the reactivation as described by Pincussen in his *in vitro* experiments. Pincussen found that when such unrayed and rayed enzyme solutions were brought together, their combined activity was considerably greater than the summation of their separate activities. Such a phenomenon may account in large degree for the results of the present experiments.

Another suggestion which might be made in regard to the stimulation of enzyme activity concerns the relations between the enzymes and their coenzymes. It might happen that the enzyme itself suffers partial inactivation by the ultra-violet rays and thus leaves an excess of coenzyme. This excess of coenzyme then stimulates the remainder of the actual enzyme to greater activity.

The experiments upon Taka-diastase and Difco invertase illustrate a point made by the author in an earlier paper (Fuller, '32), in which it was shown that at short distances from a quartz mercury vapor arc the infra-red radiation was responsible in considerable degree for injury previously attributed to ultra-violet radiation. The present experiments upon enzymes *in vitro* demonstrate that infra-red radiation causes a significant proportion of the injury to enzymes rayed in close proximity to a mercury arc, a factor which has been neglected in most of the earlier experiments.

IV. SUMMARY

1. When solutions of Taka-diastase and Difco invertase are irradiated *in vitro* by a mercury vapor arc, the enzymes suffer partial inactivation. This injury is due in part to ultra-violet, in part to infra-red radiations.

2. When bean and tomato plants are severely injured by ultra-violet rays, the activity of their amylase, invertase, peptase, and catalase is significantly increased.

3. When mycelium of *Fusarium Lini* Bolley is killed by ultra-violet rays, there is no decrease in the activity of its amylase and peptase. There is some evidence for a slight increase in the activity of these enzymes.

4. Confirmation is given earlier evidence that the death of living tissue as a result of ultra-violet irradiation is attributable to some factor other than the inactivation of enzymes, and additional data are presented to emphasize those earlier findings.

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